REMARKS

Reconsideration is requested.

A RCE was filed January 9, 2008 to ensure entry of the present Amendment and that the applicants have an opportunity to interview with the Examiner and the Examiner's Supervisor prior to the Examiner issuing a further Office Action on the merits. A separate request for an interview with the Examiner is being filed herewith to ensure that the request for an interview is not otherwise inadvertently overlooked by the Patent Office clerical and/or docketing staff.

The Examiner is requested to contact the undersigned to arrange a time convenient to the schedules of the Examiner and the Examiner's Supervisor to interview the present application.

Revision of the BIB DATASHEET in the PTO IFW to confirm that the applicants have met the requirements of 35 USC 119, and acknowledge of the same by the Examiner, are requested.

Claims 1-19 are pending. Claims 7, 11-14, 17 and 18 have been withdrawn from consideration. Rejoinder and allowance of the previously-withdrawn claims with the claims under consideration are requested.

The claims have been amended to define a "product", as described, for example, on page1, lines 11-20, and page 2, lines 24-26 of the specification. No new matter has been added.

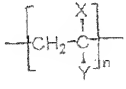
The claims are submitted to be patentable over the combined teachings of Chauvierre (WO 02/39979) and Desai (U.S. Patent No. 6,096,331). The Section 103

rejection of claims 1-6, 8-10, 15, 16 and 19 over Chauvierre and Desai is traversed. Reconsideration and withdrawal of the rejection are requested in view of the following distinguishing comments.

As the WO 02/39979 version of Chauvierre is in French, and correspondence with the USPTO is to be conducted in English, the Examiner is requested in the future to refer to U.S. Patent Publication No. 2004/0028635 A1, which is believed to be equivalent to WO 02/39979 and is printed in English. Return of an initialed copy of the attached PTO 1449 Form, pursuant to MPEP § 609, which lists U.S. Patent Publication No. 2004/0028635 A1 is requested as acknowledgement of the Examiner's consideration of the reference.

The presently claimed invention provides a product, or gas-associated form thereof, for use as blood substitute or depolluting agent comprising a hemoprotein associated with a sequenced block copolymer.

The sequenced block copolymer of the claims comprises an oligosaccharide or polysaccharide hydrophilic segment covalently linked via one of its ends to a single hydrophobic segment of formula (I), or via each of its two ends to a hydrophobic segment of formula (I), the two hydrophobic segments being the same or different;



(I)

in which:

X represents H or an alkyl, CN or CONHR radical,

Y represents a COOR', CONHR" or C_6H_5 radical,

with R, R' and R" representing, independently of one another, a hydrogen atom, a linear or branched C_1 to C_{20} alkyl group, a linear or branched C_1 to C_{20} alkoxy group, an amino acid radical, a mono- or polyhydroxylated acid radical or a C_5 to C_{12} aryl or heteroaryl radical

wherein the sequenced block copolymer is formulated as a particle whose core comprises the hydrophobic segment of formula (I), and the oligosaccharide or polysaccharide hydrophilic segment lies at the surface of the particle.

The hemoprotein of the claimed product is associated with the oligosaccharide or polysaccharide.

The sequenced block copolymer of the claimed product which comprises an oligosaccharide or polysaccharide hydrophilic segment covalently linked via one of its ends to a single hydrophobic segment of formula (I), or via each of its two ends to a hydrophobic segment of formula (I), is a compound of Chauvierre. See page 3, lines 20-29 of the present application.

The base nanoparticles of the claimed invention are made up of a sequenced block copolymer of an oligosaccharide or polysaccharide and a hydrophilic polymer of formula (I). This copolymer spontaneously forms nanoparticles where the saccharide hydrophilic segment is at the surface of the nanoparticles. This structure makes it possible to prevent the particles' uptake by the organism's nonspecific immune defense

system and, as a result, ensures the prolonged circulation thereof in the bloodstream ("stealth property").

The saccharide of the claimed invention forms a surface of the nanoparticles of the claimed invention. The saccharide at the surface of the nanoparticle of the claimed invention is associated with the hemoprotein of the claimed invention.

The saccharides of the claimed invention include dextran and heparin. The nanoparticles of the invention which include heparin therefore include a sequenced block polymer with a particle core comprising the hydrophobic segment of formula (I), and the heparin saccharide hydrophilic segment at the surface of the particle, which is in turn associated with the hemoprotein at the surface of the particle. Hemoglobin is a hemoprotein of an embodiment of the invention.

The nanoparticles of the invention include therefore a sequenced block polymer with a particle core comprising the hydrophobic segment of formula (I), and the heparin saccharide hydrophilic segment at the surface of the particle, which is in turn associated with hemoglobin at the surface of the particle.

There is no suggestion in Chauvierre or Desai to have made the claimed invention.

Contrary to the presently claimed invention, which includes an active hemoprotein associated with the external surface of the nanoparticles of the claims, Chauvierre teaches nanoparticles which are biologically active either because of the nature of the polysaccharide from which they are formed or because of incorporation of a biologically active material "in" the nanoparticles. See paragraphs [0048]-[0050],

[0052] and [0056] of US 2004/0028635 A1 and specifically, lines 1-3 of paragraph [0050], and paragraphs [0052] and [0056].

Of all of the biologically active materials described by Chauvierre as being possibly incorporated in the nanoparticles of Chauvierre (i.e., "antigens, enzymes, hormones, receptors, peptides, vitamins, minerals and/or steroids.... antiinflammatory compounds, anesthetics, chemotherapeutic agents, immunotoxins, immunosuppresants, steroids, antibiotics, antivirals, antifungals, antiparasitics, vaccinating substances, immunomodulators and analgesics.") there is no mention or suggestion of a hemoprotein of the presently claimed invention.

Chauvierre describes modification of the polysaccharide surface of the nanoparticles by "chemical functionalization" to covalently attach

> "ligands, such as targeting agents, labels or, more generally any compound capable of conferring on said particles a capability of reacting with an external species, such as, for example, a functional group on a support or a biological entity present in a medium under consideration"

to functional groups present "on the backbone of saccharide nature". See paragraph [0054] of US 2004/0028635 A1.

The hemoprotein of the presently claimed invention is not a ligand described by Chauvierre (i.e., a ligand for targeting or labeling or reacting with a support) as being covalently attached to the polysaccharide surface of the nanoparticles.

The Examiner has acknowledged that "Chauvierre et al do not teach the use of the heparin-coated poly(cyanoacrylate) nanoparticle for the delivery of hemoproteins such as hemoglobin." See page 3 of the Office Action dated July 9, 2007.

The Examiner's secondary reference, i.e., Desai, fails to cure the deficiencies of Chauvierre.

Desai provides compositions for targeted delivery of Taxol and/or Capxol, as an anti-cancer agent, to a target organ or tissue, for localization of the active drug **in** the organ or tissue. See columns 5 and 6 and specifically column 6, line 35 ("targeting of organs"), column 6, lines 47-48 ("it is very surprising that the invention formulation of paclitaxel, Capxol, a nanoparticle formulation, concentrates in tissues such as the prostate, pancreas, testes ..."), column 6, line 67 through column 7, line 3 ("A preferred embodiment of a composition to achieve high local concentrations of paclitaxel in the prostate is a formulation containing paclitaxel and albumin with a particle ..."), column 8, lines 61-64 ("It is another object of the present invention to provide a new formulation of paclitaxel that localizes paclitaxel in certain tissues, thereby providing higher anticancer activity at these sites.") .

Desai is similar to Chauvierre therefore to the extent they teach targeted delivery of a drug or active **in** to organs or tissues.

One of ordinary skill in the art will appreciate that the claimed products, which provide for example a blood substitute, will benefit from an increased circulation time. The claimed products are not designed to target organs or tissues. The claimed invention would be contrary to the aim and teaching of both Desai and Chauvierre in this regard.

Desai mentions hemoglobin in two instances.

Specifically, Desai mentions in column 9, lines 42-44 and 54 that hemoglobin is an example of a

> "suitable biocompatible material" which may be "employed in the practice of the [Desai] ... invention for the formation of a polymeric shell."

Desai also states the following in column 11, lines 60-67:

> "In other cases, the polymer forming the shell could participate in the delivery of a biologic, e.g., in the case of antibodies used for targeting, or in the case of hemoglobin, which may be delivered as part of a polymeric shell formed in the ultrasonic irradiation process described above, thereby providing a blood substitute having a high binding capacity for oxygen. "

The cited Desai patent fails to describe "the ultrasonic irradiation process described above" or further describe how hemoglobin is to "participate in the delivery of a biologic".

The cited Desai patent however is a continuation-in-part of U.S. Patent No. 5,916,596, which is a continuation-in-part of U.S. Patent No. 5,665,382. Each of the parent patents are incorporated-by-reference in the cited Desai patent.

U.S. Patent No. 5,665,382, (herein after Grinstaff) describes and claims methods of preparing pharmaceutically active agents for in vivo delivery. The claimed method of Grinstaff involves cross-linking disulfide bonds of a biocompatible material with high intensity ultrasound to form a polymeric shell of the crosslinked material which contains a pharmaceutically active agent in the polymeric shell. See claim 1 of Grinstaff for example. Claim 4 of Grinstaff specifically includes hemoglobin as a protein biocompatible material containing cross-linkable disulfide bonds which may be used to

form a polymeric shell of Grinstaff. Claim 3 of Grinstaff alternatively states that "polysaccharides containing sulfhydryl groups and/or disulfide groups" may be biocompatible materials which may be used to form a polymeric shell of Grinstaff.

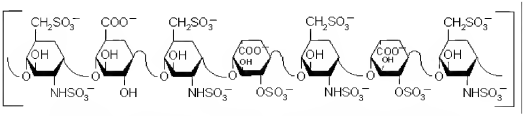
The cited Desai patent therefore, in referring to hemoglobin in the above-quoted passages of column 11 of the Desai patent, will be understood by one of ordinary skill in the art to be a reference to a polymeric shell formed of cross-linked hemoglobin. Alternatively, the cited Desai patent will be understood, from the whole of the patent and its incorporated-by-reference parent patent (i.e., Grinstaff), to relate to a polymeric shell formed of cross-linked polysaccharides containing disulfide or sulfhydryl groups.

Neither Desai nor Grinstaff teach or suggest a polymeric shell formed of cross-linked hemoglobin "coated with" (see Advisory Action dated November 23, 2007) or associated with heparin or any other saccharide or polysaccharide. Neither Desai nor Grinstaff teach or suggest a polymeric shell formed of cross-linked polysaccharides containing sulfhydryl groups associated with a protein, such as hemoglobin. The Examiner will appreciate that heparin is not a saccharide or polysaccharide containing sulfhydryl or disulfide groups[1], as would be required according to the teachings of Grinstaff and Desai to form a polymeric shell with high intensity ultrasound.

[1] Heparin is a mucopolysaccharide with a molecular weight ranging from 6,000 to 40,000 Da. The average molecular of most commercial heparin preparations is in the range of 12,000 - 15,000. The polymeric chain is composed of repeating disaccharide unit of D-glucosamine and uronic acid linked by 1->4 interglycosidic bond. The uronic acid residue could be either D-glucuronic acid or L-iduronic acid. (Structure below) Few hydroxyl groups on each of these monosaccharide residues may be sulfated giving rise to a polymer with that is highly negatively charged. The average negative charge of individual saccharide residues is about 2.3.

Moreover, Grinstaff further describes the aim and purpose of Desai's brief mention of hemoglobin polymeric shells in the following passage from column 19, line 22 through column 21, line 4 of Grinstaff (as obtained from www.uspto.gov):

> Blood substitutes described in the prior art contemplate only soluble hemoglobins as oxygen carriers. Indeed, it is conventionally accepted that an insoluble hemoglobin molecule (e.g., one that is excessively polymerized, or crosslinked with other hemoglobin molecules to the point of insolubility, or which is insoluble due to excessive denaturation, and the like) is not a candidate for reversible binding of oxygen, due to the high probability of destruction or disruption of the oxygen binding site within the molecule. In addition, the soluble hemoglobins of the prior art have Hill coefficients which are no greater than that of unmodified native hemoglobin.
>
> In contrast, polymeric shells prepared from hemoglobin, as described herein, are `giant` macroscopic molecules (due to extensive polymerization or crosslinking of large numbers of hemoglobin tetramer molecules) which, due to the large size thereof, is insoluble in aqueous medium. The polymerization occurs as a result of crosslinking of the sulfhydryl groups on the cysteine residues of the protein during the ultrasonic irradiation process. Polymeric shell prepared in accordance with the present invention typically comprises at least 10.sup.4 crosslinked polymer molecules, and may have as many as 10.sup.12 hemoglobin tetramers crosslinked into a single macroscopic `megamer` of hemoglobin. It has unexpectedly been found that oxygen can bind reversibly to these insoluble constructs with affinities that are in the useful range for a red blood cell (RBC) substitute, i.e., P.sub.50 between about 10 mm Hg to about 50 mm Hg.



http://www.people.vcu.edu/~urdesai/hep.htm#Heparin%20-%20Structure

Another surprising and unexpected observation concerning the insoluble hemoglobin construct (IHC) of the present invention is the surprisingly high Hill Coefficient (n) therefor. The Hill coefficient is a measure of the level of cooperativity between oxygen binding sites (heme units) within the hemoglobin tetrameric molecule. The maximum Hill coefficient for native hemoglobin is approximately 2.8, while Hill coefficients typically reported for prior art modified hemoglobins are less than 2.8. The measured Hill coefficients for the Insoluble Hemoglobin Constructs of the present invention are extraordinarily large, typically in the range of about 5 to about 25. Without wishing to be bound by any theory of action, these astonishingly high values can be attributed to the interaction or communication between the oxygen binding sites of the neighboring crosslinked tetrameric hemoglobin units. Essentially, it is believed that the large Hill coefficient is an indication that multiple tetramers cooperate in switching from the deoxy-T (tense) to the oxy-R (relaxed) state within the insoluble construct upon binding oxygen.

The unexpectedly large Hill coefficients observed in the hemoglobin constructs of the present invention have the advantage that the amount of oxygen carried per tetramer unit of hemoglobin far exceeds that achievable with native hemoglobin or modified hemoglobin of the prior art. This increased oxygen carrying capacity is greatly beneficial in the utility of the invention as a RBC substitute.

The hemoglobin constructs of the present invention achieve their maximum Hill coefficients at partial pressures of oxygen in the range of about 40-100 mm Hg. In other words, maximum cooperativity is achieved in this range of oxygen pressure. Since typical alveolar pO.sub.2 lies within this range, maximum uptake of oxygen from the lungs by the hemoglobin constructs will be achieved when invention constructs are utilized as a blood substitute.

On the other hand, the release of oxygen to the tissues by the invention constructs is very similar to physiological hemoglobin, i.e., at typical tissue pO.sub.2 (<40 mm Hg), most of the oxygen bound to the insoluble hemoglobin construct is released for oxygenation of the tissue. Thus, the crosslinked insoluble hemoglobin of the present invention

has the unusual ability to bind oxygen at a higher capacity (due to large Hill coefficients) than prior art hemoglobin at typical loading pressures (such as in the lungs), while retaining the ability to release oxygen efficiently at typical pressures encountered in tissue.

Due to their crosslinked nature and size, the insoluble hemoglobin constructs of the present invention are likely to have an in vivo circulation time considerably longer than red blood cell (RBC) substitutes of the prior art. Furthermore, due to their large molecular (macroscopic) size, they are not likely to induce the renal toxicity problems that are commonplace with conventional tetrameric or oligomeric soluble forms of hemoglobin described in the prior art.

The hollow (`bubble-like` or microbubble) insoluble hemoglobin constructs of the present invention may be loaded with an appropriate gas within the hemoglobin shell or membrane. Thus when the hemoglobin `microbubbles` are equilibriated with oxygen, e.g., in an external device or within the lungs, the central core of the construct or bubble is saturated with unbound or free oxygen that enters the core by molecular diffusion. Thus the constructs carry unbound molecular oxygen within their hollow core reservoir in addition to the oxygen bound to the hemoglobin forming the microbubble shell or membrane. The ability of this system to carry unbound (but entrapped) oxygen greatly increases the oxygen carrying capacity of the system over and above the oxygen carried by the hemoglobin alone. None of the prior art demonstrates this ability of carrying a reservoir of unbound molecular oxygen along with oxygen bound to hemoglobin.

Insoluble hemoglobin constructs can also be preloaded or saturated with oxygen prior to intravascular administration, for maximum oxygen delivery in short duration applications such as in coronary angioplasty or tumor therapy.

The discrete `cellular` nature of insoluble hemoglobin constructs of the present invention renders them likely to transport oxygen in a physiologic manner, not unlike red blood cells in vivo. Due to the `megameric` nature of invention insoluble hemoglobin constructs, they will have a colloidal osmotic pressure or oncotic pressure that is

> negligible compared to an equivalent amount (in terms of oxygen carrying capacity) of soluble hemoglobin of any of the prior art. This would allow for the intravenous infusion of high concentrations of invention hemoglobin constructs, while soluble hemoglobin of the prior art may be infused at a maximum concentration of only 6-8 g/dl for fear of severe water loss from tissues surrounding the vascular space due to osmotic gradients.

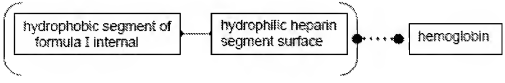
The above-quoted passage will be understood by one of ordinary skill in the art to clarify the vague reference in Desai to the use of hemoglobin polymeric shells as specific targeting or delivery agents. Specifically, Desai lists "physiologically active gasses", among the following broad genus of "biologic[s]" which may be delivered by the particles of polymeric shells (see column 9, lines 17-27 of Desai (as obtained from www.uspto.gov):

> As used herein, the term "biologic" refers to pharmaceutically active agents (such as analgesic agents, anesthetic agents, anti-asthamatic agents, antibiotics, anti-depressant agents, anti-diabetic agents, anti-fungal agents, anti-hypertensive agents, anti-inflammatory agents, anti-neoplastic agents, anxiolytic agents, enzymatically active agents, nucleic acid constructs, immunostimulating agents, immunosuppressive agents, physiologically active gases, vaccines, and the like), diagnostic agents (such as ultrasound contrast agents, radiocontrast agents, or magnetic contrast agents), agents of nutritional value, and the like.

The description in Grinstaff to the use of particles of hemoglobin crosslinked polymeric shells containing oxygen to deliver oxygen is consistent with, and further explains, the aim and intent and teaching of Desai in the only two instances where

hemoglobin is mentioned as a crosslinkable material of the polymeric shell of Desai's particles.

The Examiner is again urged to appreciate that the presently claimed invention provides a product of nanoparticles which include a sequenced block polymer with a particle core comprising the hydrophobic segment of formula (I), and a heparin saccharide hydrophilic segment at the surface of the particle, which is in turn associated with hemoglobin at the surface of the particle. This structure may be simply illustrated, without limitations, as the following linear representation of a component of the claimed nanoparticles, wherein the structure in brackets forms the nanoparticles and the hemoglobin is associated with the surface which contains the hydrophilic heparin:



In contrast to the above non-limiting schematic of an embodiment of the presently claimed invention, Desai teaches a particle shell of preferably crosslinked albumin or other disulfide or sulfhydryl containing proteins, such as hemoglobin (as further elucidated by Grinstaff which is incorporated-by-reference in Desai), which may be used as a targeting agent for chemotherapeutic drugs or encapsulated oxygen (in the case of crosslinked hemoglobin polymeric shells). Neither Desai nor Grinstaff teach or suggest nanoparticles made of polymeric shells containing a combination of a sequenced block polymer of formula (I) of the present claims covalently linked to a

saccharide, such as heparin, in a particle shell, which is associated with a hemoprotein, such as hemoglobin.

Further, there is no teaching, suggestion or motivation in the cited Chauvierre or in Desai (or Grinstaff), or any of the art of record, to make or use the claimed invention. At best, Chauvierre teaches a nanoparticle of a sequenced block polymer of formula (I) of the present claims covalently linked to a saccharide, which may be used to encapsulate active materials for in vivo delivery. Desai, at best, teaches the production of particles of crosslinked albumin, or other disulfide- or sulfhydryl-containing proteins (or disulfide- or sulfhydryl-containing saccharides such as hemoglobin) for delivery of encapsulated chemotherapeutics (or encapsulated oxygen in the case of the crosslinked "megameric" hemoglobin particles of Desai as elucidated by Grinstaff).

It is unclear to the applicants what combination one of ordinary skill would have made of the teachings of Chauvierre and Desai in the absence of the present disclosure as the cited disclosures are believed to be two distinct means of making nanoparticles for delivery of encapsulated biologically active materials. The presently claimed invention however includes an active material (i.e., hemoprotein such as hemoglobin) to be associated with the surface of the core-shell nanoparticle of the claimed invention. The presently claimed invention is contrary therefore to any common teaching of the cited art. Moreover, Grinstaff (which is incorporated-by-reference in Desai) teaches that non-crosslinked and inadequately crosslinked and prior teachings of crosslinked

hemoglobin have proved unacceptable as blood substitutes (see columns 17 and 18 of Grinstaff)[2].

The claims are submitted to be patentable over the combined teachings of Chauvierre and Desai.

For completeness, the applicants note the Examiner's statement that:

> "Desai et al disclose that hemoglobin may be present in the polymeric shell of a heparin-coated particle, thereby providing a blood substitute." See Advisory Action dated November 23, 2007.

The Examiner is requested to specifically indicate where Desai provides such a teaching. A word search of the electronic copy of Desai and Grinstaff available through the USPTO web site fails to indicate where either patent teach "heparin". Further,

[2] Stroma-free hemoglobin (SFH), taken out of the red blood cell microenvironment, has been found to exhibit a propensity to bind oxygen too tightly (a low P.sub.50) and also to have a short circulating half-life following transfusion. The low P.sub.50, reflective of a leftward shift in the hemoglobin oxygen binding curve, was, in part, a consequence of exposure of stroma-free hemoglobin to a higher pH in plasma (7.4) than that experienced within the erythrocyte (7.2); furthermore, the natural association between hemoglobin and 2,3-diphosphoglycerate was destroyed when hemoglobin was removed from the red cell, thus further lowering the P.sub.50. In terms of clearance from the circulation, stroma-free hemoglobin is observed to be rapidly eliminated by the kidneys, with a transfusion half-life (t.sub.1/2) of only about 100 minutes. The Hill coefficient for SFH is in the range of 2.3-2.8.

Chemically modified hemoglobins that address some of the shortcomings of stroma-free hemoglobin have been explored. Modifications described in the prior art include various means for intramolecular crosslinking of stroma-free hemoglobin; means for intermolecular crosslinking of stroma-free hemoglobin with low molecular weight agents; means for intra and inter molecular crosslinking of stroma-free hemoglobin with low molecular weight agents; and means for coupling stroma-free hemoglobin to other polymers.

Methods of intramolecular crosslinking of stroma-free hemoglobin are known in the art. See, for example, U.S. Pat. Nos. 4,584,130, 4,598,064 and 4,600,531. This treatment modifies stroma-free hemoglobin by covalently linking the lysine-99 residues on the alpha chains of the protein through a fumarate bridge. As a consequence of this intramolecular cross-linking, diaspirin crosslinked hemoglobin has an oxygen affinity equivalent to that of blood. Furthermore, diaspirin crosslinked hemoglobin (molecular weight 64,500) can no longer break down into dimers (molecular weight 32,250). As a result, the retention time of diaspirin alpha-alpha crosslinked hemoglobin is four to eight hours (which is two to four times that of stroma-free hemoglobin). However, this is not a sufficient length of time for utility in the treatment of acute hemorrhage, since an oxygen carrier is needed that can carry oxygen for several days when the patient has lost a considerable amount of blood. The P.sub.50 of diaspirin crosslinked hemoglobin is in the physiological range (24-28 mm Hg) as is the Hill coefficient (2.5-2.8).

neither Desai nor Grinstaff teach a particle of crosslinked hemoglobin "coated" with any saccharide or polysaccharide.

The only "coated" crosslinked hemoglobin particles taught by Desai/Grinstaff are (1) a phospholipid bilayer-coated or photopolymerizable lipid-coated, insoluble-crosslinked-hemoglobin particle which is described as making the insoluble-crosslinked-hemoglobin particle more like a red blood cell., and (2) an insoluble-crosslinked-hemoglobin particle with covalently attached polyethylene glycol (i.e., PEG). Specifically, Grinstaff teaches the following (obtained from www.uspto.gov) at column 26, line 49, through column 27, line 17, with regard to the a phospholipid bilayer-coated or photopolymerizable lipid-coated, insoluble-crosslinked-hemoglobin particle:

> In order to make the IHC in a greater likeness to red blood cells, a phospholipid bilayer can be formed around the crosslinked hemoglobin microbubbles. Such a bilayer results in the formation of a true `red cell analog` and may be created in a two step process. Charged phospholipids or lipids utilized in the formation of this bilayer include phosphatidyl choline, phosphatidyl ethanol amine, phosphatidyl serine, phosphatidyl inositol, phosphatidyl glycerol, sphingomyelin, dimyristoylphosphatidic acid, dipalmitoyl phosphatidic acid, sarcosinates (sarcosinamides), betaines, monomeric and dimeric alkyds, and the like. Nonionic lipids may also be utilized in this invention, including polyethylene fatty acid esters, polyethylene fatty acid ethers, diethanolamides, long chain acyl hexosamides, long chain acyl amino acid amides, long chain amino acid amines, polyoxyethylene sorbitan esters, polyoxy glycerol mono- and di-esters, glycerol mono- and di-stearate, glycerol mono- and di-oleate, glycerol mono- and di-palmitate, and the like.
>
> Another variation on this technique is to utilize photopolymerizable lipids or lipids that may be readily crosslinked via a chemical reaction in order to provide a more stable lipid `membrane ` coat. Photopolymerizable

> lipids that may be utilized in the present invention include acrylate or methacrylate substituted lipids (such as phosphatidyl choline, phosphatidyl ethanol amine, phosphatidyl serine, phosphatidyl glycerol, dimyristoylphosphatidic acid, dipalmitoyl phosphatidic acid, and the like); lipids with native polymerizable unsaturation (such as unsaturated phosphatidyl cholines with diacetylene groups or conjugated diene groups, and the like), and so on. Lipids that readily undergo crosslinking via thiol-disulfide exchange also are good candidates for the formation of a stable lipid coat for the IHC. Examples of such lipids include derivatives of phosphatidyl cholines esterified with lipoic acid, and the like.

Grinstaff further demonstrates an insoluble-crosslinked-hemoglobin particle with covalently attached polyethylene glycol in Example 20 of the patent. See column 44 of Grinstaff.

The Examiner is requested to specifically indicate where Desai discloses "heparin-coated particle" or a "heparin-coated particle" with hemoglobin "present in the polymeric shell", as is understood to be the Examiner's interpretation of the cited Desai.

The Examiner is further understood to believe that Desai teaches that hemoglobin "may be associated with the nanoparticle shell comprising a polysaccharide so as to be useful as a blood substitute". See Advisory Action dated November 23, 2007. As has been explained in detail above, Desai and Grinstaff teach that nanoparticles of Desai are, in one embodiment, crosslinked hemoglobin as hemoglobin contains cross-linkable sulfhydryl or disulfide groups and can be used not only to administer encapsulated oxygen but can also be used to transport oxygen in vivo as the ultrasonic crosslinking method of Desai/Grinstaff allegedly does not substantially diminish the native oxygen-exchange capacity of the hemoglobin. Desai therefore does

not describe that "hemoglobin may be associated with the nanoparticle shell comprising a polysaccharide so as to be useful as a blood substitute" as stated by the Examiner.

Further, the Examiner asserts, presumably as an alleged justification for a reasonable expectation of success in making the claimed invention from the combination of Chauvierre and Desai, that

> "the art has long recognized that heparin, being polyanionic in nature, has a high affinity for basic proteins like hemoglobin." See Advisory Action dated November 23, 2007.

The relevance of the statement is unclear. The cited art fails to teach or suggest a combination of a particle polymeric shell containing heparin which is associated with hemoglobin, as required by an embodiment of the presently claimed invention. Clarification is requested in the event the rejection of the claims over Chauvierre and Desai is maintained.

The Examiner has also criticized the applicants previously-filed remarks as allegedly not being persuasive because the applicants have allegedly argued the references individually. See Advisory Action dated November 23, 2007. The failures of the combination of cited art to teach or suggest the claimed invention are further detailed above.

The Examiner has asserted that the rejection based on Chauvierre and Desai is not an inappropriate application of hindsight reconstruction. Specifically, the Examiner states the following:

> But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge

> gleaned only from the Applicant's disclosure, such a reconstruction is proper. See In re McLaughlin, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971).
> In the instant case, the Examiner has used only the teachings of the prior art as the basis for the rejections. See Advisory Action dated November 23, 2007.

As noted above however, each of the cited references describes polymeric particles for delivering encapsulated active agents. Moreover, the effect of altering the structure of Chauvierre was unpredictable from the cited art. Moreover, it is believed to have been contrary to Desai to produce a polysaccharide-containing particle associated with hemoglobin on it's surface as Desai teaches the advantages of a crosslinked hemoglobin particle per se and describes only a limited number and types of "coating" or attached moieties which may be applied to the crosslinked hemoglobin particle (i.e., phospholipid bilayer-coated or photopolymerizable lipid-coated and PEG attached).

The applicants submit, with due respect, that the Examiner has included, in the hindsight application of the cited art, knowledge "gleaned only from the Applicant's disclosure". The cited combination of art is not believed to establish a prima facie case of obviousness.

Further, as noted above, the polysaccharides of Desai/Grinstaff contain disulfide or sulfhydryl groups. Heparin does not include disulfide or sulfhydryl groups and neither Desai nor Grinstaff describe heparin as a saccharide of their invention.

As previously described in the applicants Remarks of November 9, 2007, Chauvierre teaches, and one of ordinary skill in the art will appreciate, that the surface properties of core-shell nanoparticles prepared according to Chauvierre, can vary greatly depending on the polymerization method. As taught by Chauvierre, anionic

polymerization of an alkylcyanoacrylate in presence of a polysaccharide such as dextran leads to branched copolymers that self associate into nanoparticles that are rapidly taken up by the macrophages of the Mononuclear Phagocyte System (MPS). Their lifetime in vivo is therefore reduced. In contrast, when free radical polymerization is used, sequenced copolymers are obtained that associate into nanoparticles where the polysaccharide chains are arranged as a brush at the nanoparticle surface. It is this "brush-like" structure that confers to the nanoparticles the long circulating life that is essential for their application as blood substitutes. There was no reasonable or predictable expectation of success from the cited references, or from the general knowledge in the art, that the surface properties of the nanoparticles, in particular their long- circulating life in blood, would not be negatively impacted if they were associated with hemoglobin. The inventors demonstrated as much in the previously-submitted Chauvierre et al. (Cell. Molec. Biol., 2004, 50(3), 233-239) and the cited Desai, as elucidated by Grinstaff, is believed to further highlight the unpredictability of hemoglobin-containing blood substitutes. Specifically, Grinstaff is believed to teach the importance of highly cross-linked hemoglobin polymer particles which is not required by and would be contrary to the presently claimed invention.

Similarly, there was no reasonable or predictable expectation of success that the hydrodynamic radius of these nanoparticles would not be negatively affected by association of hemoglobin at their surface. The inventors demonstrated that the size of nanoparticles containing heparin was not significantly affected by the association of

hemoglobin (see previously-submitted Chauvierre et al, Cell. Molec. Biol., 2004, 50(3). 233-239; Chauvierre et al, Biomaterials, 2004, 25, 3081-3086).

Finally, there was no reasonable or predictable expectation of success that the associated hemoglobin would retain its capacity of transporting gases such as oxygen or carbon monoxide. Again, the inventors demonstrated that hemoglobin was functional (see Chauvierre et al, Cell. Molec. Biol., 2004, 50(3), 233-239) and the cited Desai, as elucidated by Grinstaff, is believed to further highlight the unpredictability of hemoglobin-containing blood substitutes.

The applicants further submit that the quantities of hemoglobin associated at the surface of the nanoparticle may vary greatly depending on the nature of the polysaccharide present at the surface of the nanoparticle and/or depending on whether the copolymer is branched ("loop like" structure) or sequenced ("brush-like" structure). The following examples illustrate this principle:

	Polysaccharide	Nanoparticle type and structure	Hemoglobin loading capacity (mg/ml)
1	Dextran (70 kDa)	PIBCA – "loop-like" structure"	0.3
2	Dextran (70 kDa)	PIBCA – "brush-like" structure	0.8
3	Dextran sulfate (10 kDa)	PIBCA – "brush-like" structure	1.2
4	Dextran sulfate (40 kDa)	PIBCA – "brush-like" structure	1.9
5	Heparin (19 kDa)	PIBCA – "brush-like" structure	2.7

PIBCA=polyisobutylcyanoacrylate

As the data shows, everything else being equal, there is a nearly three fold difference in hemoglobin loading capacity between nanoparticles prepared by free

radical mechanism (sequenced copolymer — "brush-like" structure) and nanoparticles prepared by the classical method (branched copolymer — "loop-like" structure) [1 and 2]. The polysaccharide molecular mass [3 and 4] as well as the nature of the polysaccharide [2 and 5] also affect the hemoglobin loading capacity. Finally, the presence of functional groups on the polysaccharide is also relevant [entry 2 with entries 3 and 4; and entries 2 and 5]. The above results were not reasonably predictable from the cited art.

The applicants have previous argued that Desai failed to provide enabling support for making particles of crosslinked hemoglobin and that Desai is inoperable in this regard. The Examiner has not been persuaded by these remarks and has directed the applicants attention to In re Sasse, 207 USPQ 107 (CCPA 1980) and In re Donohue, 226 USPQ 619 (Fed. Cir. 1985). See the Advisory Action dated November 23, 2007. Citation to Grinstaff, as explained above, which was only recently appreciated by the undersigned during the preparation of the present Amendment, would appear to more directly address the issue.

The claims are submitted to be patentable over the cited combination of art. Withdrawal of the Section 103 rejection is requested.

The claims are submitted to be in condition for allowance and a Notice to that effect is requested. The Examiner is requested to contact the undersigned, preferably by telephone, in the event anything further is required.

An interview with the Examiner and the Examiner's supervisor is requested prior to the Examiner's next Action on the merits.

Respectfully submitted,

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